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THE MANURING OF CENTROSEMA PUBESCENS

BY

R. A. HAMILTON AND K. S. PILLAY

Summary

The results are given of two pot-experiments with Centrosema pubescens growing in a typical granite soil taken from a field under mature rubber trees.

In the first experiment, where nodulation was poor and little or no nitrogen fixation occurred:—

(a) Nitrogen applied in the form of sulphate of ammonia depressed growth and nodulation but it increased the nitrogen content of the plants.

(b) Phosphorus applied as superphosphate considerably increased growth; potassium slightly increased growth; smokehouse ash depressed growth of plants.

In the second experiment basic slag gave by far the best results, and amongst the rock phosphates Gafsa phosphate was outstanding whilst Christmas Island phosphate appeared to be inferior to the other phosphates; superphosphate gave disappointing results, the heavier dressings appearing to depress both growth and nitrogen fixation.

The importance of nodulation and nitrogen fixation in legumes, particularly when they are grown as cover plants in association with rubber trees, is clearly seen. In the absence of nitrogen fixation the legumes compete with other plants for available soil nitrogen.

The opinion is held amongst many rubber planters in Malaya that leguminous covers are more suitable than natural covers for rubber estates. Accordingly renewed efforts are being made to establish leguminous covers under mature rubber trees, a process which has not so far been very successful in Malaya, except in isolated cases, although in other rubber-growing countries it appears to offer less difficulty.

A wealth of information is available from other countries regarding the manurial requirements of legumes but this may only be of limited application under Malayan conditions, and since manuring is one of the most important factors in establishing and maintaining leguminous covers under mature rubber trees, a number of experiments were begun in 1937 with a view to obtaining information regarding the manurial requirements of legumes under Malayan conditions. The results of two pot-experiments with *Centrosema pubescens* are recorded in the present paper.

Many legumes do not tolerate acid conditions whereas others grow well in acid soils. Experience in Malaya suggests that *Centrosema pubescens* belongs to the second group; in one field experiment on a soil of pH of 4.5, the application of lime appeared to retard the growth of this legume.

It is universally agreed that a liberal supply of phosphate is necessary for satisfactory growth of legumes. Phosphate not only increases the size of the plant but also the size and number of root nodules, and it has been reported by some workers to have increased the nitrogen content of the plant.

As regards nitrogen, most of the published evidence suggests that nitrogen applied in the form of nitrates or ammonium salts is undesirable as it retards, and in some cases prevents, the formation of root nodules.

In general it has been found that applications of potash slightly improve the growth of legumes, but with the exception of soils markedly deficient in potash, most soils grow legumes successfully without the addition of potash.

Experiment No. 1

Manurial Treatments

The treatments used in this experiment comprised all the possible combinations of three levels of nitrogen (N), phosphate (P) and potash (K) in the presence and absence of smokehouse ash (A), giving a total of 54 treatments. The rates of application of the fertilisers calculated as cwt. per acre were as follows:

Sulphate of ammonia (20% nitrogen)	N_0 = Nil N_1 = $2\frac{1}{2}$ cwt. N_2 = 5 „
Superphosphate (18% phosphoric acid)	P_0 = Nil P_1 = $7\frac{3}{4}$ cwt. (=1.4 cwt. P_2O_5) P_2 = $15\frac{1}{2}$ „ (=2.8 „ P_2O_5)
Sulphate of potash (48% potash)	K_0 = Nil K_1 = $1\frac{1}{4}$ cwt. K_2 = $2\frac{1}{2}$ „
Smokehouse ash (12% potash)	A_0 = Nil A_1 = 5 cwt.

The soil used in the experiment was a typical granite loam taken from a field under mature rubber trees, chemical analysis having indicated that it was deficient in nitrogen and phosphorus and slightly deficient in potassium. The pots used in the experiment were nine inches high and glazed on the inside. The soil was diluted with river sand to prevent packing and the manures thoroughly mixed with the soil. The pots were automatically watered from below and in addition water was periodically added to the top. *Centrosema pubescens* seeds were inoculated by sprinkling them with a water-suspension of a Rhizobium culture and allowed to germinate in sand, and after a week seedlings were transferred to each of the 54 pots. The pots were placed in an open plant shed under partial shade; after a fortnight the shade was removed daily between 8 a.m. and 10.30 a.m. The pots were placed at random in the shed and moved to a new position every third day. After a fortnight each pot was watered with 100 c.c. of a water-suspension of Rhizobium.

Observations on Growth

Some of the seedlings suffered from die-back and these were replaced by healthy seedlings. This was especially the case with the pots manured with sulphate of ammonia. The detailed observations made at various stages of growth may be summarized as follows:—

1. Nitrogen (N) depressed growth and the depression was greatest with the heavy dressing.

2. The leaves of the plants in the N_0 pots were yellow and typical of plants suffering from nitrogen deficiency, while those in the N_1 and N_2 pots were dark green in colour. This indicated that little or no nitrogen fixation was taking place in the N_0 pots.

3. Phosphate (P) increased growth considerably but in the absence of nitrogen produced a very pale yellow leaf.

4. Potash (K) increased growth slightly in the absence of ash but had no appreciable effect in the presence of ash.

5. Smokehouse ash (A) retarded growth considerably in the early stages, and although this effect was considerably reduced at eight weeks, it was still evident.

The condition of the plants at eight weeks in typical pots can be seen from Plate I.

Plate I

A = $N_1P_0K_0A_1$ D = $N_0P_1K_0A_0$

B = $N_2P_0K_2A_0$ E = $N_0P_2K_0A_0$

C = $N_0P_0K_0A_0$ F = $N_2P_2K_2A_0$

G = $N_1P_2K_1A_0$

The plants were harvested complete with roots at eight weeks, when the roots were examined for nodulation and the plants were dried, weighed and the percentage of nitrogen, phosphoric acid and potash in the dry matter determined. The results are summarised in Table I.

INTERACTIONS

1. The effect of P on the phosphoric-acid-content of the plants was greater in the absence than in the presence of ash.

2. In the presence of A, K depressed growth but in the absence of A, K increased growth.

3. The depressing effect of K on the nitrogen content of the plant was greater in the absence than in the presence of A.

4. K increased the potash content of the plant more in the absence than in the presence of A; and conversely for A; indicating that both K and A supply potash with the usual diminishing response to increasing levels.

TABLE I

Effects of N, P, K and A on the dry weight and total nitrogen produced per pot and the percentage nitrogen, phosphoric acid and potash in the dry matter

Treatment	N_0	N_1	N_2	S.E.
Dry weight of plants per pot (gms.)	7.58	7.38	6.90	±0.203
Weight of nitrogen per pot (mgs.)	127	163	199 s	—
% nitrogen in plants ...	1.74	2.36	3.04 s	±0.095
% phosphoric acid in plants ...	0.46	0.52	0.55 s	±0.0120
% potash in plants ...	1.98	1.96	1.95	±0.061
Treatment	P_0	P_1	P_2	
Dry weight of plants per pot (gms.)	5.05	8.34	8.47 s	
Weight of nitrogen per pot (mgs.)	145	174	185 s	as
% nitrogen in plants ...	2.87	2.08	2.19 s	above
% phosphoric acid in plants ...	0.40	0.53	0.60 s	
% potash in plants ...	2.02	1.95	1.92	
Treatment	K_0	K_1	K_2	
Dry weight of plants per pot (gms.)	7.15	7.16	7.56	
Weight of nitrogen per pot (mgs.)	185	173	162 s	as
% nitrogen in plants ...	2.58	2.42	2.14 s	above
% phosphoric acid in plants ...	0.51	0.51	0.51	
% potash in plants ...	1.63	1.99	2.27 s	
Treatment	A_0	A_1		
Dry weight of plants per pot (gms.)	7.81	6.76	s	±0.166
Weight of nitrogen per pot (mgs.)	186	161	s	—
% nitrogen in plants ...	2.38	2.37		±0.078
% phosphoric acid in plants ...	0.49	0.53	s	±0.0098
% potash in plants ...	1.83	2.10	s	±0.049

S.E. = Standard Error of the values

Significant effects marked thus: s

s indicates $P<.01$

NODULATION

An examination of the roots for nodulation showed that (1) nodules were in general small and sparse, (2) they were best on the plants supplied with phosphate and (3) they were poorest on the plants supplied with nitrogen.

DISCUSSION OF RESULTS

The results in general agree with previous work in confirming the pronounced beneficial effect of phosphate and less pronounced effect of potash, and the depressing effect of nitrogen on nodulation. Furthermore, they show that the level of the phosphate dressings was needlessly high, since there was very little difference between the effect of the single and double dressing. The depressing effect of nitrogen on growth is difficult to explain. It may be that the high concentration of sulphate of ammonia gave the seedlings an initial set back and had the experiment been extended for a longer period this effect might have disappeared. At the end of the experiment the plants supplied with nitrogen appeared to be in a much more healthy condition than those without N.

The depressing effect of smokehouse ash was probably due to the fact that it was fresh and strongly alkaline, the lime (CaO) content being about 30 per cent. The figures for percentage potash in the leaf show that the smokehouse ash can act as a satisfactory supply of potash, the average content for fresh ash being about 10 to 20 per cent K_2O. It is possible that had a similar dressing of an older ash been used the beneficial effects of the potash might have been obtained without the depressing effect of the ash. This points to the importance of exercising care in the use of smoke-house ash as a manure for leguminous covers.

The sparsity of nodules was disappointing and considerably influenced the results of the experiment. The culture used was shown from laboratory tests to be in good condition and the failure in the experiment was probably owing to the method of inoculation. The failure of the method of inoculation, however, serves a useful purpose in emphasising the importance of nodulation and nitrogen fixation in leguminous plants, particularly when used as cover plants in conjunction with other crops. In the absence of nodulation and nitrogen fixation they feed on, rather than add to, the soil nitrogen and in this way seriously compete with rubber tree roots.

Experiment No. 2

Experiment No. 1 showed that on the soil used in that experiment phosphate is the most important manure for *Centrosema*

pubescens and experiment No. 2 was designed to obtain further information relative to type and quantity of phosphate.

Treatments

The treatments used in this experiment comprised four levels (P_1, P_3, P_5 and P_7) of each of the following phosphatic fertilisers:

	per cent P_2O_5
Superphosphate	18
Basic Slag	17
Gafsa rock phosphate	24
Safaga rock phosphate	30
Cheribon rock phosphate	29
Christmas Island rock phosphate	37
Egyptian rock phosphate	30

The phosphates were applied on an equal P_2O_5 basis, and the amounts applied, calculated as cwt. P_2O_5 per acre, were as follows:

P_1 = 0.35 cwt. P_2O_5 per acre

P_3 = 1.05 cwt. „ „ „

P_5 = 1.75 cwt. „ „ „

P_7 = 2.45 cwt. „ „ „

The treatments were replicated in two blocks giving a total of 56 pots.

The technique and general experimental conditions were similar to those of experiment No. 1 with the following exceptions:

1. *Inoculation.* The seed was inoculated by the milk-phosphate method and the culture suspension applied to each pot contained 0.05 gms. calcium di-hydrogen phosphate.

2. A small basal dressing of potash was applied to all pots.

Observations on Growth

The growth was in general better than in experiment No. 1 even for those pots of the latter which received phosphate. Basic slag and superphosphate gave the best early growth.

At four weeks after planting it was observed that the plants were very pale and were obviously suffering from nitrogen deficiency, but after six weeks some of the new leaves were dark green and much larger than the older ones, and dark patches appeared on some of the older pale leaves. This became general at about eight weeks, and it was then clear that nitrogen fixation was taking place and that the plants were being supplied with nitrogen through the nodules. This afforded a very striking demonstration of the importance of nitrogen fixation.

The following observations were made just before harvesting:

1. Basic slag gave by far the best growth and the leaves were larger and of a darker green than in any of the other treatments.

2. Gafsa rock phosphate was outstanding amongst the other treatments but there was no noticeable difference between the other phosphates except that Christmas Island appeared to give the poorest growth, while the leaves were paler on the superphosphate-treated plants than on any others.

The condition of typical plants eight weeks after planting can be seen from Plate II.

Plate II

1. Basic slag
2. Gafsa rock phosphate
3. Superphosphate
4. Cheribon rock phosphate
5. Egyptian rock phosphate
6. Safaga rock phosphate
7. Christmas Island rock phosphate

(All pots of the P_5 level)

TABLE II

Effect of treatments on the dry weight of the complete plants, dry weight of nodules only, weight of nitrogen in complete plants, weight of nitrogen in underground portion and weight of P_2O_5 in above ground portion of plants

Type of Phosphate	Level of P_2O_5				
	P_1	P_3	P_5	P_7	Mean
Dry weight of complete plants (Gms. per pot)					
Basic Slag ...	17.8	20.4	23.2	22.9	21.1
Gafsa ...	12.7	18.5	19.6	18.2	17.3
Cheribon ...	12.0	13.9	17.4	17.5	15.2
Egyptian ...	12.4	16.2	14.2	17.7	15.1
Safaga ...	10.7	16.9	14.2	14.9	14.2
Superphosphate ...	12.8	17.7	16.9	15.1	15.7
Christmas Island ...	12.4	11.9	14.8	15.9	13.8
Standard Error ...	± 1.594				± .797
Dry weight of nodules (Mgs. per pot)					
Basic Slag ...	334	563	571	623	523
Gafsa ...	241	381	439	492	388
Cheribon ...	111	149	334	355	237
Egyptian ...	128	255	224	400	254
Safaga ...	94	281	232	331	234
Superphosphate ...	145	253	236	164	200
Christmas Island ...	119	118	224	276	186
Standard Error ...	± 70.5				± 35.2
Mgs. nitrogen per pot (complete plants)					
Basic Slag ...	362	534	513	598	502
Gafsa ...	216	365	449	402	358
Cheribon ...	199	251	353	360	290
Egyptian ...	191	285	381	390	312
Safaga ...	183	341	270	326	280
Superphosphate ...	202	329	267	252	262
Christmas Island ...	186	196	274	263	230
Standard Error ...	± 56.1				± 28.1
Mgs. nitrogen per pot (Underground portion only)					
Basic Slag ...	86	90	108	111	99
Gafsa ...	58	74	82	90	76
Cheribon ...	50	57	72	77	64
Egyptian ...	48	72	60	76	64
Safaga ...	45	73	59	67	61
Superphosphate ...	56	68	67	47	59
Christmas Island ...	54	51	67	66	59
Standard Error ...	± 11.15				± 5.57
Mgs. phosphoric acid per pot (Above ground portion only)					
Basic Slag ...	40	57	64	76	59
Gafsa ...	22	36	40	38	34
Cheribon ...	24	36	50	44	38
Egyptian ...	24	30	38	40	33
Safaga ...	37	46	44	46	43
Superphosphate ...	33	40	55	61	47
Christmas Island ...	24	30	34	39	32

The plants were harvested at nine weeks, dried and weighed and the percentage of nitrogen and phosphoric acid in the dry matter determined. A separate weighing was made of the dried nodules.

The results are summarised in Table II.

Statistical analysis of the above figures shows that the effect of basic slag is greater than that of Gafsa, which in turn is greater than those of the remaining phosphates in respect of each of the following characters:—

1. Dry weight of plants
2. Dry weight of nodules
3. Weight of nitrogen in complete plants
4. Weight of nitrogen in roots.

As regards the phosphorus content of the plants, this is greatest in the basic slag treatments and the plants manured with superphosphate and Safaga are higher in phosphate than the remainder.

Discussion of Results

The outstanding feature of the experiment is the marked superiority of basic slag over all the other treatments, basic slag supplying *three* parts of P_2O_5 giving much better returns than any of the other phosphates supplying *seven* parts P_2O_5. This can be clearly seen by an examination of Figures 1 to 4. It may be that had the experiment been carried out over a longer period the effect of the rock phosphates would have improved relatively to that of basic slag, but this seems unlikely in view of the fact that, with the exception of Gafsa phosphate, the difference between basic slag and the other phosphates increased with time.

This advantage of basic slag may be due to its more easily available phosphate content and also to its alkaline nature. Further, it is likely that a high proportion of essential minor elements are present and available to the plants. These possibilities were not investigated and they are mentioned as probable explanations of increased effect from basic slag.

Gafsa phosphate appears to be a good substitute for basic slag and the results indicate that Gafsa supplying *five* parts of P_2O_5 gave considerably better results than any of the other rock phosphates supplying *seven* parts of P_2O_5. Although there is

no significant difference between the effects of the remaining rock phosphates, Christmas Island phosphate appears to have given consistently poorer results than the others, particularly in respect of those characters which are influenced by Rhizobium activity. This can be seen from Table III.

TABLE III

Comparison of mean effect of Christmas Island and other Rock Phosphates (Christmas Island taken as 100)

	Christmas Island	Safaga	Cheribon	Egyptian	Gafsa
Weight of nodules ...	100	128	130	136	211
Weight of N per pot	100	121	126	135	156

It should be noted that Christmas Island phosphate is more coarsely ground than the others. This probably explains the poorer results in these short-term experiments. When used in the field, where its action can extend over months instead of weeks, the difference between this and other phosphates may well be much less.

The effect of superphosphate is peculiar in that the heavier dressings appear to depress all characters except the phosphorus content of the plants, whereas in the case of all the other phosphates all characters are increased by increasing dressing. (Figs. 1 to 5). The most probable explanation of this (if it is a real effect: see Appendix) is that the relatively high concentration of the sulphate ion is either in itself toxic or causes a transient increase in acidity which may either affect the plant or the activity of the Rhizobium or both.

Obviously for commercial procedure the cost of the fertilisers must be considered and where growth effects are not vastly different or are only delayed in appearing, then differences between the types of phosphatic fertilisers can be ignored and the choice of fertilisers can be based on the cost per unit of phosphoric acid. Closely related to cost also is the ease with which the fertilisers can be procured, and this is of paramount importance under war-time conditions.

Belfast, September 1940.

FIG 1

THE EFFECT OF PHOSPHATES ON DRY WEIGHT OF PLANTS

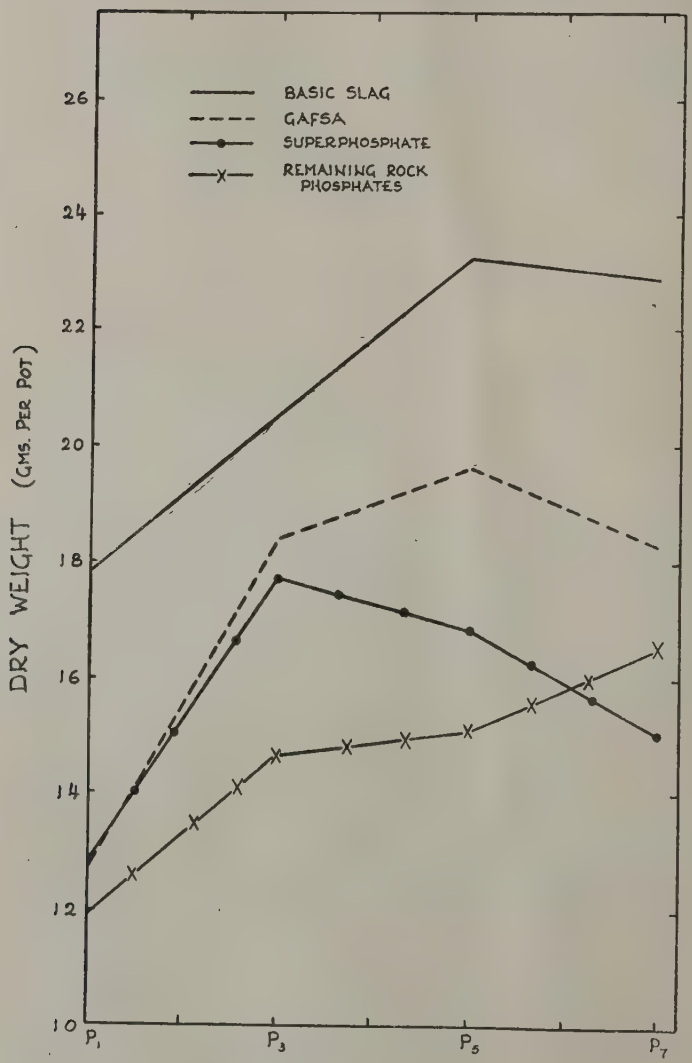


FIG 2

THE EFFECT OF PHOSPHATES ON DRY WEIGHT OF NODULES

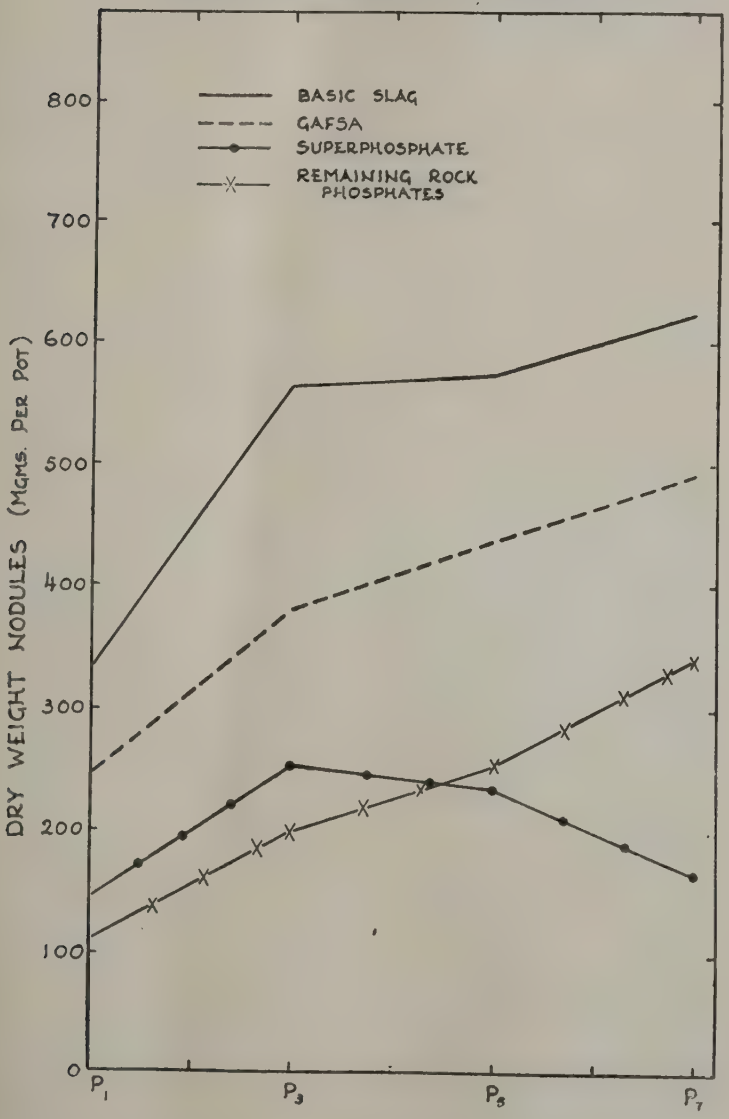


FIG 3

THE EFFECT OF PHOSPHATES ON THE WEIGHT OF NITROGEN IN THE COMPLETE PLANTS

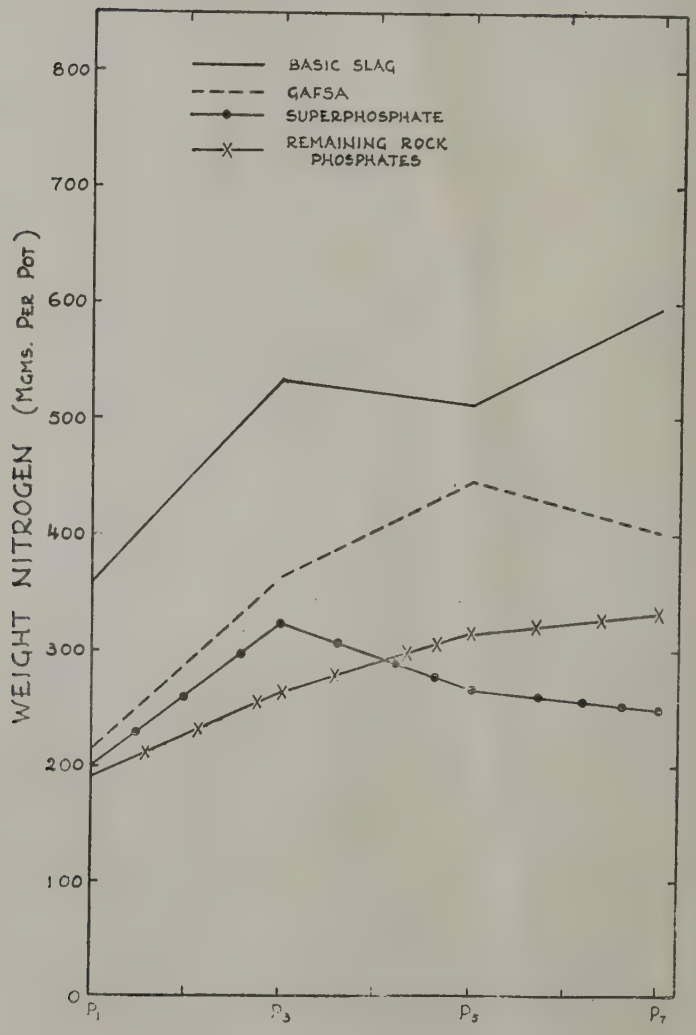


FIG 4

THE EFFECT OF PHOSPHATES ON THE WEIGHT OF NITROGEN IN THE ROOTS AND NODULES

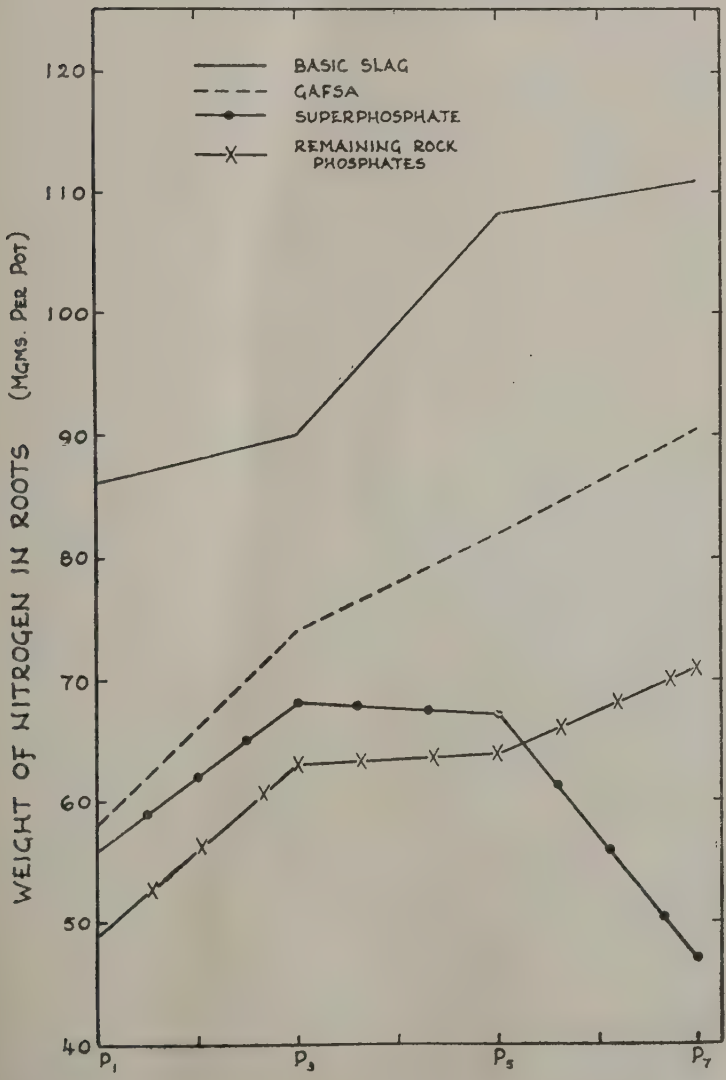
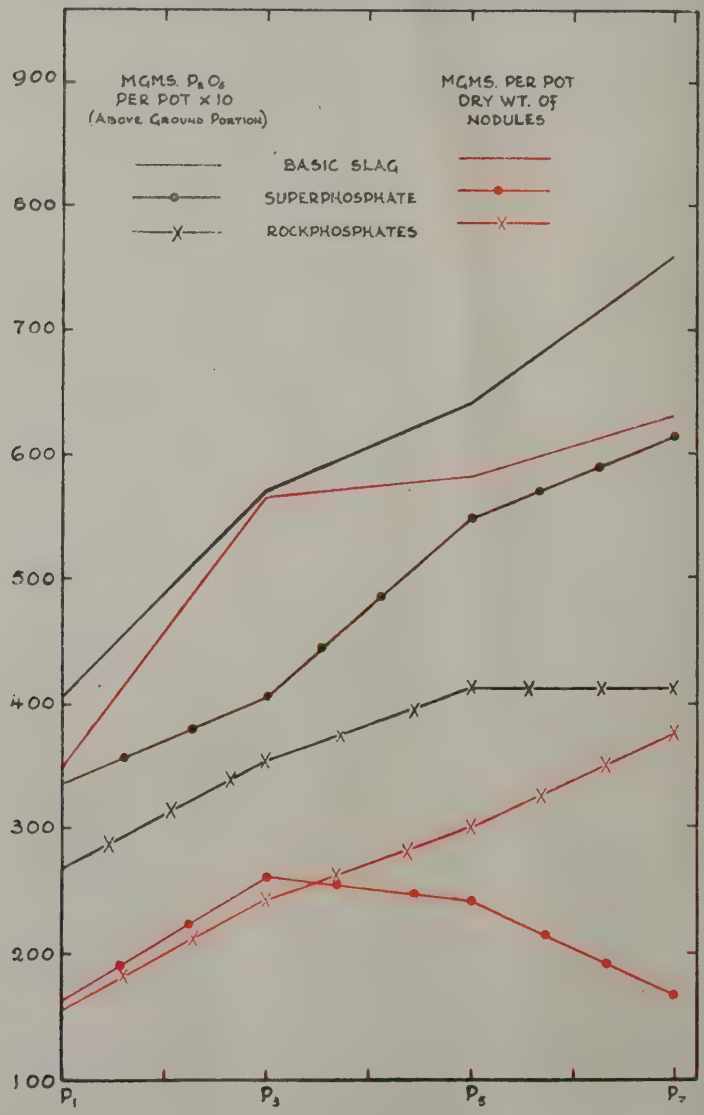


FIG 5

A COMPARISON BETWEEN THE EFFECTS OF THE PHOSPHATES ON THE PHOSPHORIC ACID CONTENT OF THE PLANTS AND THE FORMATION OF ROOT NODULES



Statistical Appendix

BY

H. Fairfield Smith

Readers familiar with the design and analysis of factorial experiments may find the following statement of treatment effects etc. useful to summarise the evidence and its reliability.

Experiment I		D.Wt. gm.	% N	% P_2O_5	% K_2O
Linear Responses *					
(1) A	...	**−1.06**	**− .01**	**.046**	**.28**
(2) N′	...	*− .69*	**1.30**	**.090**	**−.03**
„ P′	...	**3.43**	**− .68**	**.202**	−.10
„ K′	...	.41	**− .45**	−.005	**.65**
Curvatures †					
(3) N″	...	*− .27*	.05	−.034	.02
„ P″	...	**−3.16**	**.89**	*−.068*	.04
„ K″	...	.38	− .11	.011	−.08
Significant Interactions ‡					
(2) AN′	...	—	**− .37**	**−.047**	—
(3) AN″	...	**1.72**	—	—	—
(2) AP′	...	—	—	**−.073**	—
(2) AK′	...	**−1.14**	*.32*	—	−.17§
(3) AK″	...	—	—	—	.30§
(4) N″K′	...	*−1.60*	—	—	—
Standard errors					
Rows (1)	...	.234	.110	.0138	.0699
(2)	...	.287	.135	.0170	.0856
(3)	...	.498	.234	.0294	.1482
(4)	...	.608	—	—	—

Bold face type indicates significant at the 1% level
Italic type indicates significant at the 5% level

§ Individually these two interactions show P slightly greater than .05, but taken together are significant at the 5% level.
Standard errors estimated from 28 d.f.

* "Linear Response" = difference between observations at double and zero level, e.g. $(n_2 - n_0)$ etc.

† "Curvature" = difference of response to second unit over response to first unit, e.g. $(p_2 - 2p_1 + p_0) = (p_2 - p_1) - (p_1 - p_0)$ etc.

‡ "Interaction" = one half of the difference in response (or of curvature) to one factor in presence and absence of another e.g. the % N in presence and absence of ash and double nitrogen was as follows:—

		a_0	a_1
n_0	...	1.63	1.84
n_2	...	3.31	2.77

Response to n_2 (=N′) in presence of ash = 2.77 − 1.84 = .93
Response to n_2 (=N′) in absence of ash = 3.31 − 1.63 = 1.68
One half of difference = AN′ = −.37

Experiment II

Analyses of Variance

	d.f.	Mean Squares D. Wt. (gm.)	Nodules D. wt. (mgm.)	N in roots mgm.	N in plant mgm.	N %
Qualities ...	6	**49.173**	**120.297**	**1649**	**64,613**	**.5161**
Levels: Linear ...	1	**140.581**	**311.289**	**2766**	**161568**	**.9676**
Quadratic ...	1	*36.806*	13.578	330	*32835*	.0480
Cubic ...	1	4.325	6.625	28.9	2715	.2532
Linear Regressions × Qualities	6	2.243				
Quadratic „ × „	6	5.475	7.025	149.0	4045	.0595
Cubic „ × „	6	5.591				
Blocks ...	1	2.240	*56.071*	*1245*	10478	**.6429**
Error ...	27	5.078	9.930	248.6	6282	.06566

The chief point to note from the above table is that we cannot, from available evidence, demonstrate differences among the qualities in slope or curvature of responses to increasing levels. In fact it appears that differences in response to qualities are arithmetically as great at the lowest as at higher levels. (A similar effect was recently observed by F. Crowther, Emp. J. Expt. Agric.9: 125-136, for response of cotton to varying levels of calcium nitrate and ammonium sulphate.) It is not unreasonable to suppose, as suggested by Hamilton, that superphosphate at the higher levels may have been harmful to nodulation, and thence to growth and nitrogen accumulation; but so far as present evidence goes this can only be accepted as a suggestion for further investigation, it is not here proven. (The figures given in Table II, and the points on the graphs for superphosphate, are each based only on 2 pots with a standard error for dry weight of ± 1.59 gm.)

A further point of interest shown by the above analysis is that although position in the shed has shown no detectable effect on dry weight, it has affected the weight of nodules and per cent of nitrogen produced.

In the light of the analyses of variance it does not seem profitable to investigate further interactions of levels × qualities, and the only statistics of interest are the means for each quality at all levels, which are shown in the last column of Table II, and the means for each level over all qualities, which are as follows:—

	p_1	p_2	p_3	p_7	s.e.	P′	P″
D. Wt. gm.	13.0	16.5	17.2	17.5	± .602	**1.42** ± .269	*− .810* ± .301
Nodules d. wt. mgm.	168	286	323	373	± 26.6	**66.7** ± 11.9	− 15.6 ± 13.3
N in roots mgm.	57	69	73	76	± 4.21	**6.28** ± 1.88	− 2.43 ± 2.10
N in plant mgm.	220	328	358	370	± 21.2	**48.0** ± 9.47	*− 24.2* ± 10.6
N %	1.66	1.96	1.90	2.08	± .0685	**.118** ± .0305	− .029 ± .034

P' = linear regression coefficent
on units of .7 cwt. P_2O_5 = $\frac{1}{10}(3p_7 + p_5 - p_3 - 3p_1)$

P'' = quadratic regression coefficent
on units of .7 cwt. P_2O_5 = $\frac{1}{4}(p_7 - p_5 - p_3 + p_1)$

Levels of significance indicated as above.

Nitrogen percentage tends to be positively correlated with dry weight of plant, both within and between treatments. But even after exhaustive analysis of covariance of the two characters it does not seem possible to reach from the data of this experiment any conclusion as to whether or not the qualities of phosphate have influenced the nitrogen percentage independently of yield.

Kuala Lumpur,

6th October, 1941.